The Universal Code Equivalent of the Mitochondrial 1-Sce | Gene.

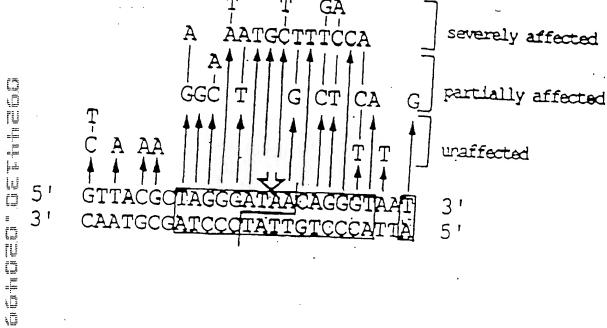
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The synthetic I-Scal gene

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1 and 2: These amino acids are absolutely necessary to produce catalytic activity. Other substitutions are possible, such as deletions of the 10 first amino acids.

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THE PROPERTY OF THE PROPERTY O 1747 TRATAGRECITETECCUTTTCCCCACCTCCACCTCCACCTCCACTTTCCCC AND CITE OTO ACC CAC CAC CAC 18 C 8 8 A 7 2 2 C A 1 3 . 1816 IPM MAY CYL CLE CLE LOC LOC LOC LOW LICENSETTANCE LANGUE CONTRACTOR 1944 APPROCATION CONTRACTOR CONTR 1006 con care ten ececunicaciativ and inv nintericaciativativamentalecontributation and 2229 ACC ATG AFF ACT LAT TOT CAT OFF TOA CARCTTATCATCRATARGOTTER AND USE TAG TYPATCAG 2195 1 2296 AGTTANATITICTANCOCACTICAGOCACTITICT ATO ANA TOT AND AAT OOD OTO ATO STO ATO CTO GOL 2363 I s 2364 ACC GTC ACC CTG CAT GCT GTA GGC ATA GGC TTG GFF AFG GGG GTA CTC CCC GGC CTC TTG 2423 זהה כבה קדב קבה כבה אנת לבו החץ מכו הרה פחץ הבו חים נות נות נות הבה הבה בבה בבה בבה בהם בבה בהם בבה בהם בבה בהם מוא מות באר המה מכא זמנ כאם כאב אום דוכ זמנ זכנ כבו אום מדם כדם אכל ככם מכת באה מין בוצון C Q D X 2 1944 MET ONE CAT USE OUT GIT CAC AAT TAA TOATCOOCTICHTATA ATC TOT COA ATT STE MEE ONA 1866 V Quit 2007 THE CHATTCHCACAGOMICASCATTE ATO CAT ATO AND ME ATE MA MA ME CAG GIA ATE M I K K 1871 THE CITE OUT COO AND TOT ANA CITE CHE MA CHA THE ANA THE CHE CITE ATE CHA CITE AND 2:10 2731 AEC SAA CIAC TITY CAA COA COT ATC COT CTC ATC CT0 est GAT ECT TAC ATC CGT TUTY COTT 2750 ROPEAGICLIL 4 9 12 2791 GAS GAA GOT ANA ACC THE TET ADD CHE THE GAE TOO ANA AND ANA OCA THE ADD CHE CAC 2800 2 6 1 7 1 CXOFITX I r 2851 OTA TOT CITE THE CAT CHE TIME OTA CITE TOT COST CHE CHE ANA ANA CHA COT OTT AND 2510 2511 CAC CTG COT AMO CTG GEA ATC ACC TOO COC GCC CAC ACT THE ANA CAC CAA CCT THE AMO 11970 IIVG Q T T X R Q 1 2971 ANA CTO GCT AND CTO THE ATE STT AND AND ANA ANA ACE ATE CCC AND AND CTO GTT GAA. 1010 H X K 7 Date that the red cost and left cle cor the fact rid and eve cut cell cell that left call in the 133 # T & T F 8 5 L A Y 1 7 ٥ c 3091 THE MAR MAN AND THE MIC AND AND TOG ATO GEA ONG AND AND CHE TOT TO ACT TTO CAA 3150 LOS Y Y X X S 2181 CAA CEA CAA TAC CTO CET AAG GET COS CET AND AAA THE CAA CTG AAC TOT TAC CTA AAA 1213 BYLVEGLES Q L 1311 ATC AAC MAA AAC MAA COS ATC ATC TAC ATC SAT STOT ATG TOT TAC UTG ATC TTC TAC AAC 3210
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I-SceI coding sequence of pSCM525 - Note the two amino acid N-terminal extension as compared to genuine version of the gene.

VARIATIONS AROUND THE 1-SCR ! SECUENCE

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Parities than can be changed without affecting encome activity (demonstrated) positions -1 and -2 are not natural. The two amino acids are added due to cloning strategies

positions 1 to 10: can be deleted

position 36: G is tolerated

position 40: M or Y are tolerated

position 41: 8 or H are talcrated

position 43: A is tolerated

position 44: Yor Mare interated

position 91: A is telerated

positions 133 and 156: Lare tolerated position EER A and Sare tolerated

Changes that office engage activity (demonstrated)

position 19: Land

position 35: I to S or N

position \$9: Gts Dor R

provision 40: L to 2"

position 42 Lt. R

position 44: Dio E Gor H

pesition 45: A to E or D

president 46: Y to D

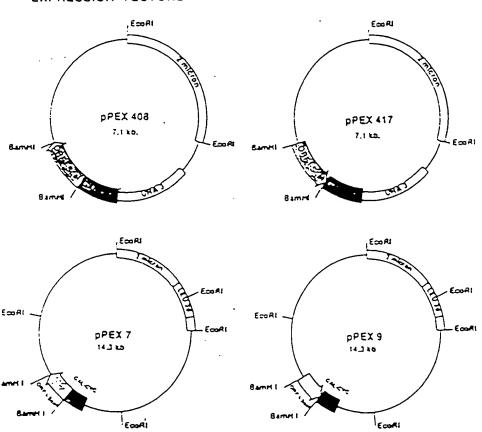
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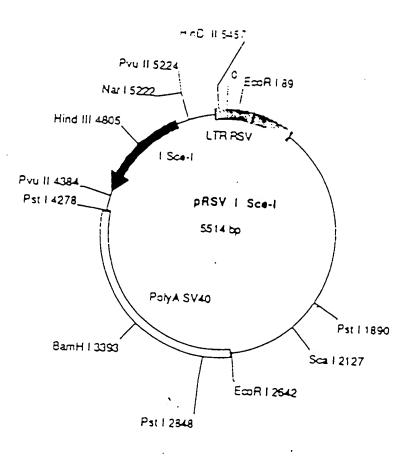
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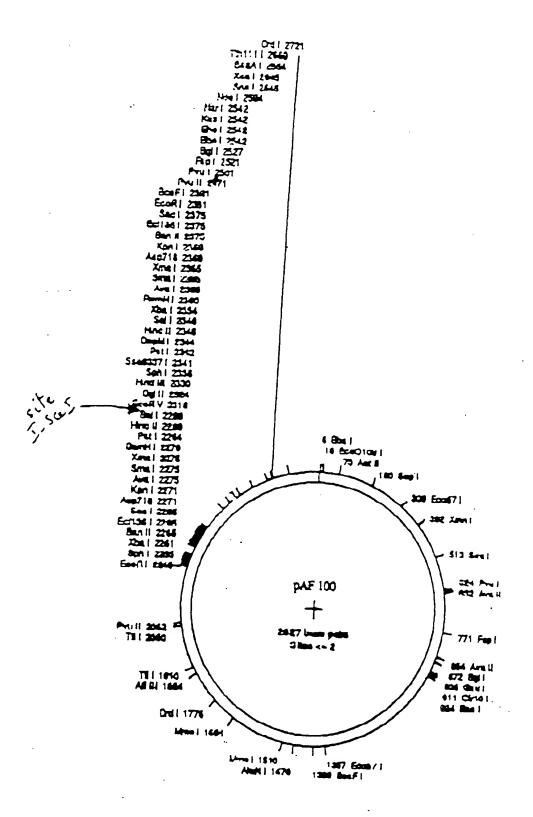
Group I Intron Encoded Endonucleases and Related Endonucleases

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ten)	l-for II (Sectheromyces mitochondria)	SAAACCAGTAGGTAGTA	: 2 : 4 : : : : :
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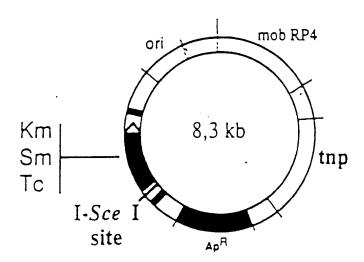
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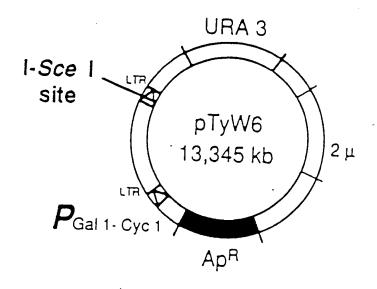
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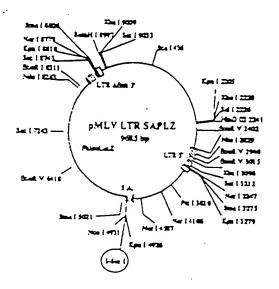


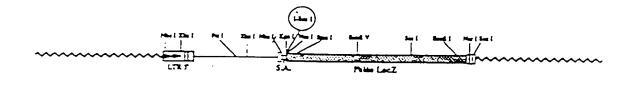
Construction: pGP 704 from De Lorenzo, with transposase gene and insertion of the linker [I-Scel] in Notl unique site

1.7



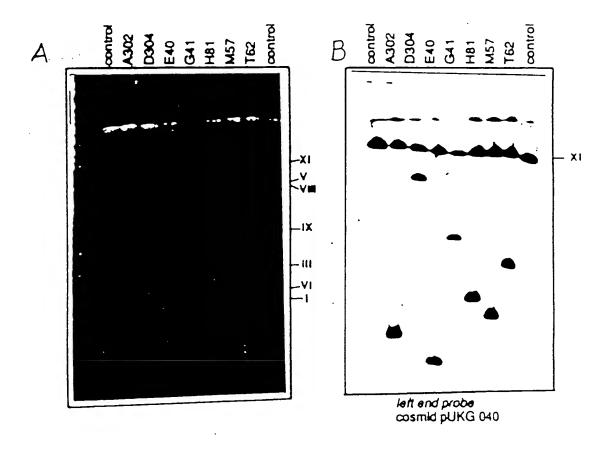
Construction: pD 123, from J. D. Boeke with insertion of a linker [I-Scel - Notl] in BamHI





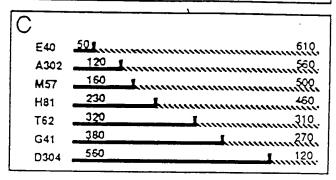
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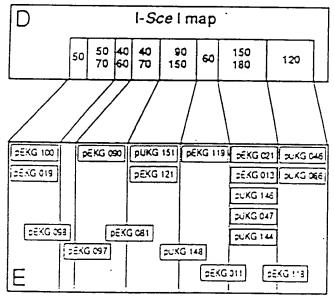
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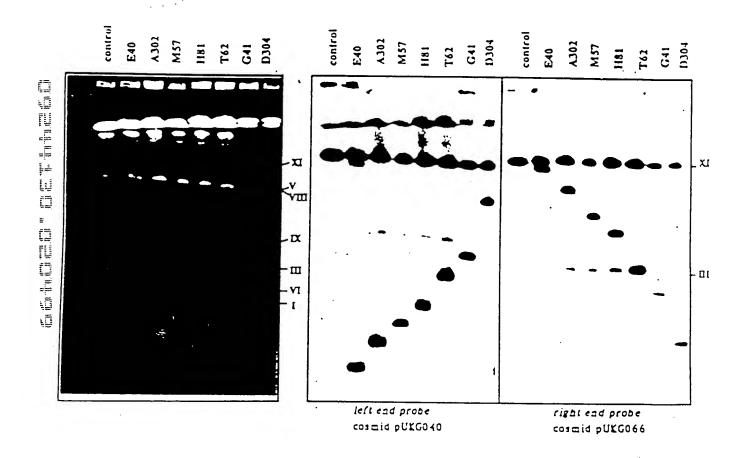


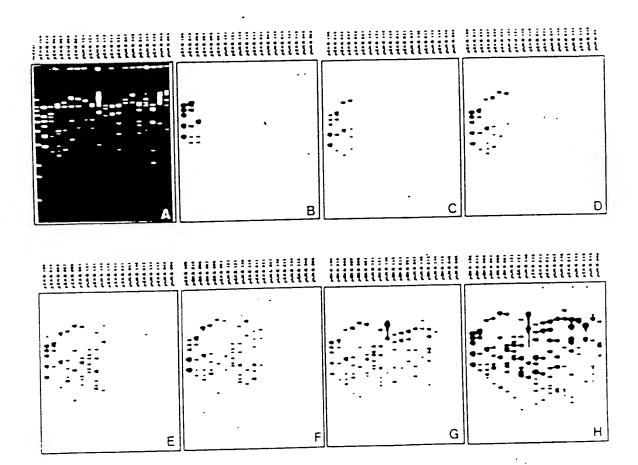
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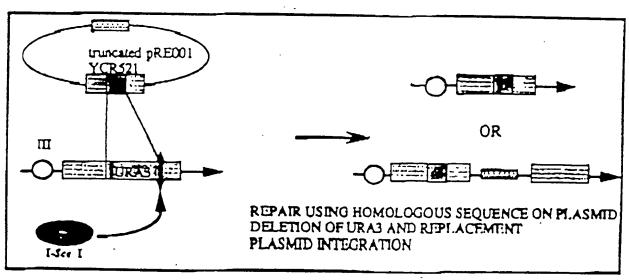


Figure 20

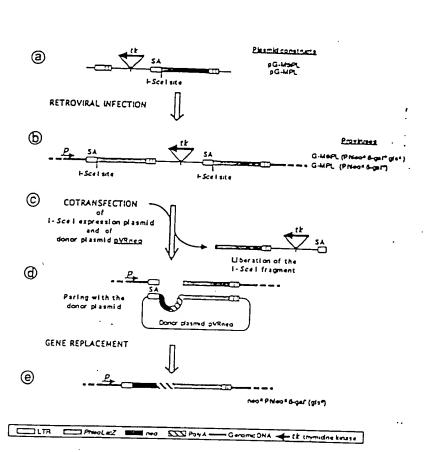


Figure 21

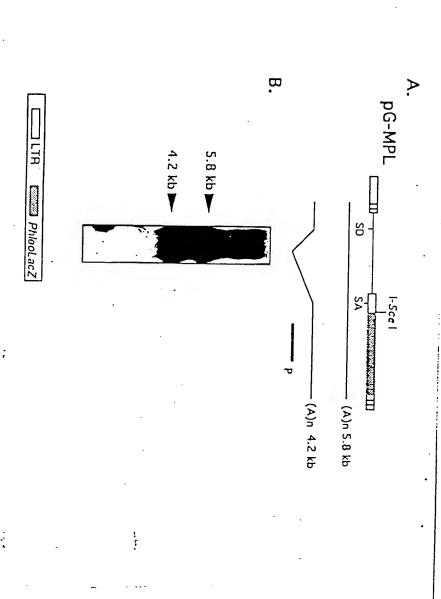
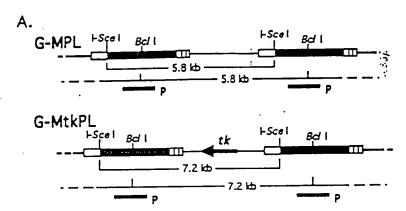


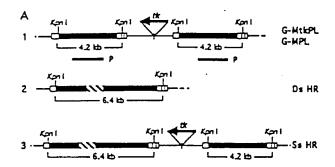
Figure 22

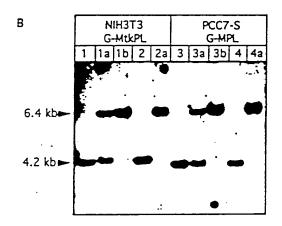


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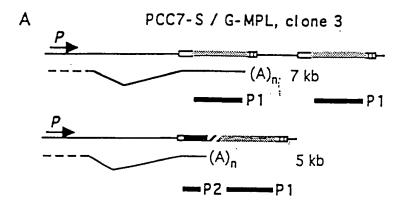
tk thymidine kinase

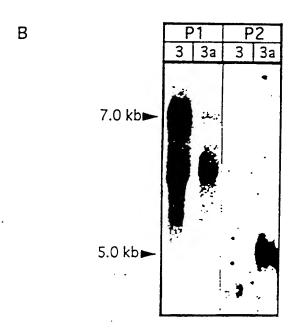


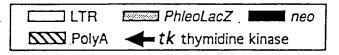


LTR PhiloDacZ PolyA — Genomic DNA — £k thymodine kinase

Figure 24





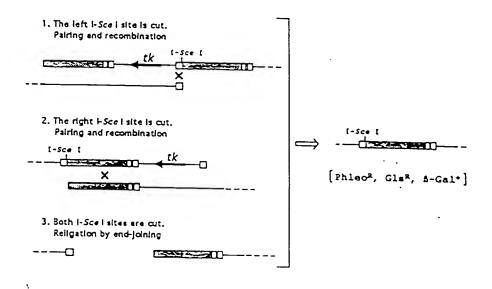


a. Chromosomal DNA containing provirus

Phenotypes

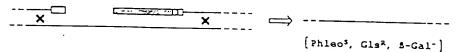


b. Intra-chromosomal recombinations events



C. Inter-chromosomal recombination event

Both I-See I sites are cut. Gap repair using intact chromosome sequences



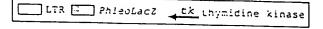
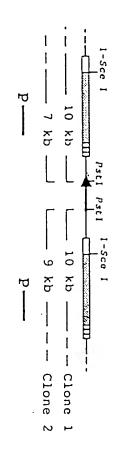


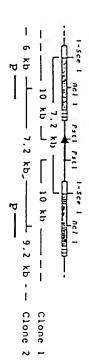
Figure 26

a. Parental DNA, G-MtkPL



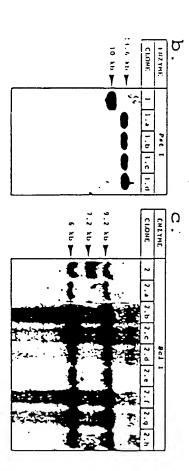
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7 X X Y Y	CLONE	ENZYME	G.
	2 2.1 2.2 2.3 2.4 2.5 2.6	Pat I	

1. Parental DNA, G-MtkPL



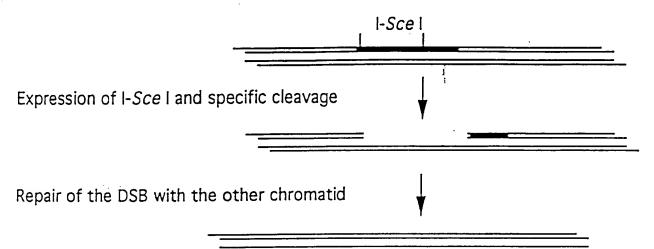
2. Intra-molecular recombination event

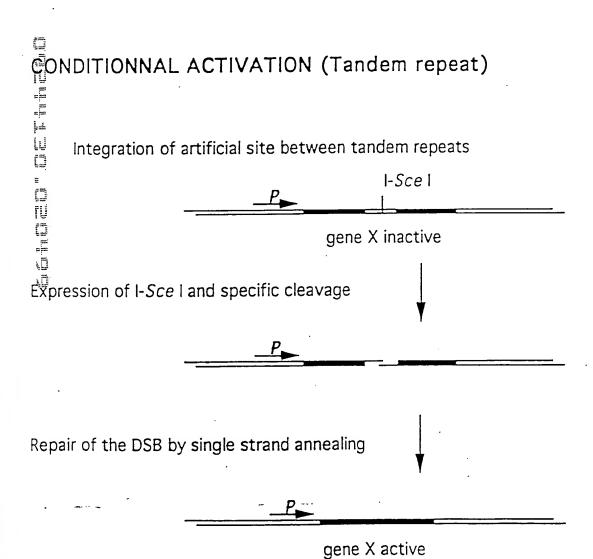
-- 6 kb -1- 9.2 kb ---Clone 1 Clone 2



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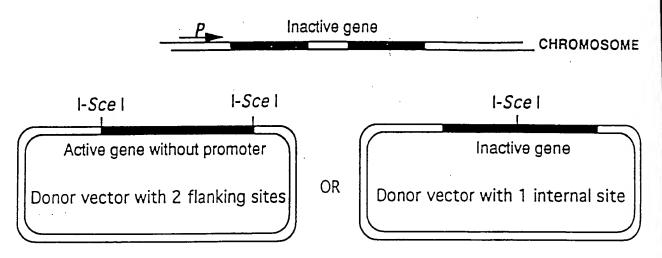
Integration of artificial site or presence of specific site



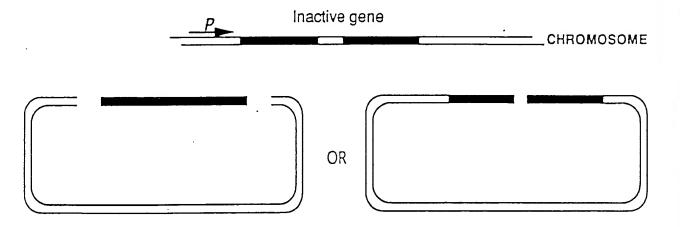




Integration of artificial site or presence of specific site



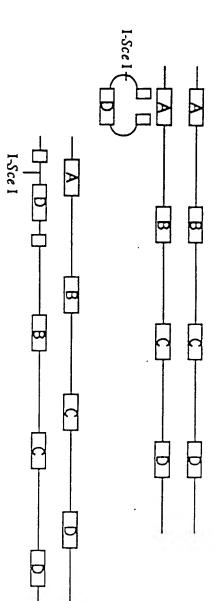
Expression of I-Sce I enzyme and specific cleavage of the donor plasmid



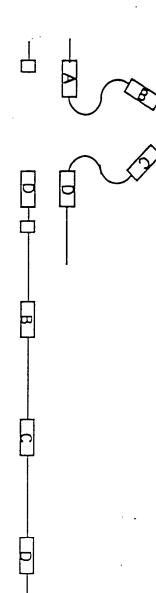
Recombination between the chromosome and plasmid



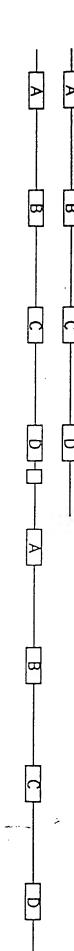
I insertion of I-Sce I site by classical gene replacement



Specific cleavage by I-Sce I enzyme and repair of the break by homologous sequences

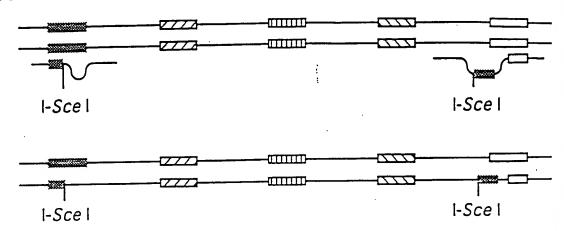


3 Duplication of the totality of the locus

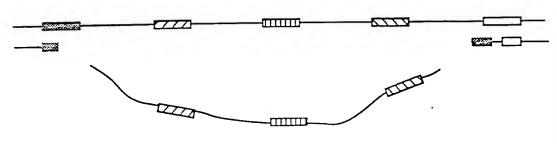




1 Insertion of two I-Sce I sites flanking the locus



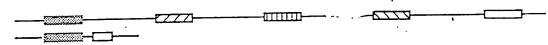
2 Expression of the enzyme and cleavage



3 Recombination between the two ends



4 deletion of the locus



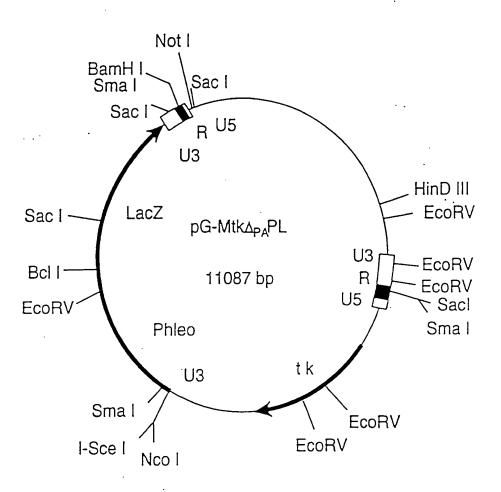


FIG. 33